36. (Amended) The set of specific binding pairs of claim 35 wherein said sensitizer is capable of generating singlet oxygen when photoactivated.

REMARKS

Claims 21, 24, 25, 27, and 30-36 have been amended and claims 23 and 29 have been cancelled. Claims 21-22, 24-28, and 30-36 are currently pending in the application. All pending claims are set forth in Exhibit A with amendments shown (if applicable).

Applicants gratefully acknowledge the 14 February 2003 interview with the Examiner in which all pending rejections in the application were discussed. During the interview, the Examiner agreed that the present amendments have addressed the concerns raised over new matter.

Applicants note (1) that, in claim 30, the term "sensitizer"in reference to the second reagent has been removed in response to the Examiners concern, and (2) that claim 36 remains pending, as express support for the claim is found in claim 7 on page 69, lines 15-16, of the specification.

Amendments have been made to clarify terms and, wherever possible, to adopt terms and language expressly set forth in the specification.

Bases for the amendments are as follows:

Claim(s)	Term/Phrase	Basis
21, 27, 30, 35	"target-binding moiety"	Page 8, line 34; Page 32, lines 20-21.
21	"that is cleaved by oxidation" in reference to the cleavable linkage.	Page 19, line 10, ("cleaved based on oxidation") of parent application 09/698,846.* Page 19, line 14 ("subject to oxidation with release of the eTag reporter" in reference to a cleavable linkage) of parent application 09/698,846.*
21, 30	"comprising a detection group, D, and a mobility modifier, M,"	Page 4, lines 21-25.
30-36	"specific binding pairs"	Page 8, line 34. Page 32, lines 20-21. Table 4 on page 33.
30, 35	"selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals" in reference to active species.	Page 18, line 27, of parent application 09/698,846.*
30, 35	"second reagent"	Page 18, line 22, of parent application 09/698,846.*

* Parent application 09/698,846 was incorporated by reference (see page 1, lines 5-8, of the specification), and the indicated passages were expressly incorporated by the 22 July 2002 Amendment.

No new matter has been added by the amendments. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. 112

In paragraph 4 of the Office Action, the Examiner rejected claims 21-36 under 35 U.S.C. 112 first paragraph because the following phrases in the claims were not found in the specification at the locations indicated in the prior amendment: (i) "antibody binding compound," (ii) "cleavable linkage is selected from the group consisting of olefins, thioethers, sulfoxides, or selenium analogs of thioethers or sulfoxides," (iii) "antibody binding compound is a monoclonal antibody or polyclonal antibody," (iv) "reagent pairs," (v) "first reagent" and "second reagent" in reference to specific binding pairs, and (vi) "second antibody binding compound having a sensitizer for generating an active species."

In view of the amendments, Applicants submit that the concerns raised by the Examiner over new matter have been addressed and respectfully request that the above rejection be withdrawn.

Rejections Under 35 U.S.C. 103

In paragraph 6 of the Office Action, the Examiner rejected claims 21-22, 26-28, 30-31, and 33-35 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski et al (U.S. patent 4,331,590) in view of Giese (Anal. Chem., 2: 166-168 (1983)). The Examiner argues as follows: Bocuslaski discloses ligands attached to indicator moieties each containing an enzymatically cleavable linkage, such that when the cleavable linkage is broken the indicator moiety becomes optically distinguishable from indicator moieties having intact linkages. Giese discloses sets of releasable electrophoric tags that may be attached to binding compounds and used in binding assays to detected multiple analytes in a single reaction. Released electrophoric tags are identified after separation by gas chromatography with electron capture detection. From Giese's teaching of the potential usefulness of multiplexed assays, one of ordinary skill in the art would have been motivated to combine the assays of Bocuslaski with the multiplexing and releasable tag of Giese to obtain Applicants' invention.

Applicants respectfully disagree, particularly in view of the amendments. Applicants' invention employs *electrophoretic separation* of eTag reporters whereas Giese employs *gas chromatographic separation* of electrophoric "release" tags. This is an important difference as the

gas phase separation of Giese limits the nature of the released tags that can be used and requires that additional steps be performed prior to separation.

In regard to the additional steps to implement Giese's method, for measurements in the disclosed binding assays, Giese's method requires that "release" tags be extracted from the assay mixture into a volatile organic solvent, then concentrated by evaporation prior to injection into a gas chromatograph (page 167, col. 2, last sentence in first full paragraph). This is a time consuming and labor intensive step that is not required by Applicants' method. In accordance with Applicants' method, *released eTag reporters can be separated by electrophoresis directly from an assay reaction mixture.*

In regard to the nature of tags that can be separated by gas chromatography, Applicants direct the Examiner's attention to the following excerpt from a description of gas chromatography on the Agilent, Inc. website:

> "It is estimated that 10-20% of the known compounds can be analyzed by GC. To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapor phase at 400-450°C or below, *and they do not decompose at these temperatures*, the compound can probably be analyzed by GC."(www.chem.agilent.com/cag/cabu/whatisgc.htm) (copy attached as Exhibit B). (Emphasis added).

Many optically active molecules, such as organic fluorescent molecules, are not available for use in Giese's method because such molecules decompose rather than volatilize at temperatures necessary for gas chromatography (e.g. rhodamine B decomposes at 211°C, carboxyfluorescein decomposes at 275°C, and the like, data from Aldrich catalog). This is not a limitation in Applicants' invention because electrophoretic separation takes place in aqueous conditions that (by definition) must be less than boiling temperature, i.e. less than 100°C.

Applicants submit that neither Bocuslaski nor Giese diclose or suggest alone or in combination the electrophoretic separation of molecular tags, such as eTag reporters. In fact, Applicants submit that Giese teaches away from the combination with Bocuslaski because the latter reference teaches the use of fluorescent organic molecules as labels and this class of labels are generally not amenable to gas chromatographic analysis. (Applicants note that conventional detectors in gas chromatographs are based on thermal conductivity, flame ionization, electron capture, or mass spectrometry, e.g. Harris, "Exploring Chemical Analysis," 2nd edition (Freeman, San Francisco, 2001), none of which are suitable for organic fluorescent molecules.) Therefore, application of the multiplexing taught by Giese, which depends on gas chromatography, would not

have been obvious to one of ordinary skill practicing the single-plex fluorescent labels of Bocuslaski.

Furthermore, in regard to claims 30-31, and 33-35, neither Bocuslaski nor Giese disclose or suggest a *second reagent* that is capable of generating an active species for cleaving the cleavable linkage.

For the above reasons, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In paragraph 7 of the Office Action, the Examiner rejected claims 23, 25, and 36 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski (cited above) in view of Giese (cited above) and further in view of Breslow et al (U.S. patent 6,331,530). Breslow discloses a compound for cancer therapy consisting of cyclodextrin dimers whose cyclodextrin pairs are connected by a linkage which is cleaved by singlet oxygen generated by a photosensitizer. The purpose of Breslow's compound is to create a locally elevated concentration of photosensitizers in a region (e.g. by a tumor) of illumination. The Examiner applies Bocuslaski and Giese as above and further cites Breslow for its disclosure of a sensitizer used to generate the active species, singlet oxygen, for cleaving a chemical bond. The Examiner argues that one of ordinary skill in the art would have been motivated to employ the sensitizer-based cleavage system of Breslow because its use in cancer therapy suggests that it is highly efficient and would therefore be applicable to the analytical assays of Giese and Bocuslaski.

Applicants respectfully disagree, particularly in view of the amendments. Applicants submit that the combination of Giese and Bocuslaski are inappropriately applied as discussed above, and that the teachings of Breslow do not correct this deficiency with respect to 23, 25, and 36. In fact, assays based on Bocuslaski and Giese as argued by the Examiner would more likely lead one of ordinary skill in the art away from Breslow because the photosensitizers of Breslow add complexity to an otherwise simple assay. For example, Giese teaches the use of cyanogens bromide for cleavage of his "release" tags and Bocuslaski teaches enzymatic cleavage of the indicator molecule. Both of these approaches involve only the addition of a single reagent. On the other hand, the system of Breslow requires (1) the addition of a photosensitizer, (2) a means for illuminating the photosensitizer, and (3) the selection of either an indicator molecule or a "release" tag that would not be affected by the chemically active singlet oxygen. Furthermore, the advantage of using the sensitizer-based cleavage taught by Breslow (enhance the localized tumor-killing affects of singlet oxygen—col. 3, lines 21-22) is not a factor that would motivate one of ordinary

skill in the art to apply it to an analytical assay. Accordingly, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In paragraph 8 of the Office Action, the Examiner rejected claims 24 and 32 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski (cited above) in view of Giese (cited above) and futher in view of Kameda et al (U.S. patent 4,780,421). Kameda discloses the use of cleavable linkages in specific binding assays, e.g. immunoassays, to permit the release of a labeling moiety, e.g. a fluorescent label, from a binding complex in order to obtain an improved signal. The Examiner argues that one of ordinary skill in the art would have been motivated to use the cleavable linkages of Kameda (which include linkages cleavable by oxidation) in the assays of Giese and Bocuslaski because they lead to increased sensitivity and are easy to apply.

Applicants respectfully disagree, particularly in view of the amendments. Applicants submit that the combination of Giese and Bocuslaski are inappropriately applied as discussed above, and that the teachings of Kameda do not correct this deficiency with respect to claims 24 and 32. Accordingly, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In paragraph 9 of the Office Action, the Examiner rejected claim 29 under 35 U.S.C. 103(a) as being unpatentable over Bocuslaski (cited above) in view of Giese (cited above) and futher in view of Bocuslaski et al (U.S. patent 4,383,031). The Examiner applied Bocuslaski ('590) and Giese as applied above and cited Bocuslaski ('031) for its disclosure of a linkage cleavable by an esterase used in an analytical assay.

Applicants respectfully disagree, particularly in view of the amendments. Applicants submit that the combination of Giese and Bocuslaski are inappropriately applied as discussed above, and that the teachings of Kameda do not correct this deficiency with respect to claim 29 Furthermore, in view of the amendment canceling claim 29, the Examiner's concern in regard to Bocuslaski ('590) and Giese in view of Bocuslaski ('031) has been obviated. Accordingly, Applicants respectfully request that it be withdrawn.

In view of the above, Applicants submit that the claims as written fully satisfy the requirements of Title 35 of the U.S. Code, and respectfully request that the rejections thereunder be withdrawn and that the claims be allowed and the application quickly passed to issue.

If any additional time extensions are required, such time extensions are hereby requested. If any additional fees not submitted with this response are required, please take such fees from deposit account **50-2266**.

Respectfully submitted,

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Exhibit A
Amendments to the Specification and/or Claims showing Insertions and Deletions

21. (Amended) A probe set for detecting the presence or absence of one or more target compounds, the probe set comprising a plurality of electrophoretic probes selected from the group defined by the formula:

$$[(D, M)\text{-}L]_k\text{-}T$$

wherein:

T is a target-binding moiety [~~an antibody binding compound~~] specific for a target compound;

k is an integer in the range of from 1 to 20;

L is a cleavable linkage that is cleaved by oxidation;

D is a detection group;

M is a mobility modifier consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, sulfur, nitrogen, phosphorus, and boron;

and wherein, upon cleavage of L an eTag reporter comprising a detection group, D, and a mobility modifier, M, is produced with a distinct charge/mass ratio so that eTag reporters from different electrophoretic probes form distinct peaks upon electrophoretic separation.

22. The probe set of claim 21 wherein said plurality is in the range of from 5 to 100, and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

23. (Cancelled) [~~The probe set of claim 21 wherein said cleavable linkage is photolabile~~.]

24. (Amended) The probe set of claim 22 wherein said cleavable linkage is [~~cleavable by oxidation and is~~] selected from the group consisting of olefins, thioethers, sulfoxides, and selenium analogs of thioethers or sulfoxides.

25. (Amended) The probe set of claim 24 wherein said cleavable linkage is cleavable [~~by oxidation~~] by singlet oxygen [~~or hydrogen peroxide~~].

26. The probe set of claim 25 wherein said detection group is a fluorophore, a chromophore, or an electrochemical label.

27. (Amended) The probe set of claim 26 wherein said target-binding moiety [~~antibody binding compound~~] is a monoclonal antibody or a polyclonal antibody; and wherein k is in the range of from 1 to 3.

28. The probe set of claim 27 wherein said charge/mass ratio is in the range of from -.001 to 0.5, and wherein said detection group is a fluorescein.

29. (Cancelled) [~~The probe set of claim 21 wherein said cleavabe linkage comprises an ester linkage that is cleavable by an esterase.~~]

30. (Amended) A set of [~~reagent~~] specific binding pairs for detecting the presence or absence of one or more target compounds, the set comprising a plurality of pairs of first reagents and second reagents, the first reagent and second reagent of each pair being specific for the same target compound, the first reagent of each pair being selected from the group defined by the formula:

$$[(D, M)\text{-}L]_k\text{-}T$$

wherein:

T is a target-binding moiety [~~an antibody binding compound~~] specific for a target compound,

k is an integer in the range of from 1 to 20,

L is a cleavable linkage,

D is a detection group, and

M is a mobility modifier consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, sulfur, nitrogen, phosphorus, and boron,

wherein upon cleavage of L an eTag reporter comprising a detection group, D, and a mobility modifier, M, is produced with a distinct charge/mass ratio so that eTag reporters of different electrophoretic probes form distinct peaks upon electrophoretic separation; and

the second reagent of each pair [~~comprising a second antibody binding compound having a sensitizer for~~] being capable of generating an active species to cleave the cleavable linkage, the active species being selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals.

31. (Amended) The set of [~~reagent~~] specific binding pairs of claim 30 wherein said plurality is in the range of from 5 to 100, and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron.

32. (Amended) The set of [~~reagent~~] specific binding pairs of claim 31 wherein said cleavable linkage is selected from the group consisting of olefins, thioethers, sulfoxides, and selenium analogs of thioethers or sulfoxides.

33. (Amended) The set of [~~reagent~~] specific binding pairs of claim 32 wherein said detection group is a fluorophore, a chromophore, or an electrochemical label, and wherein said charge/mass ratio is in the range from -.001 to 0.5.

34. (Amended) The set of [~~reagent~~] specific binding pairs of claim 33 wherein said target-binding moiety [~~antibody binding compound~~] is a monoclonal antibody or a polyclonal antibody, and wherein k is in the range of from 1 to 3.

35. (Amended) The set of [~~reagent~~] specific binding pairs according to claims 30, 31, 32, 33, or 34 wherein said second reagent [~~antibody binding compound~~] is a monoclonal antibody or a polyclonal antibody, and wherein said active species is selected from the group consisting of singlet oxygen, hydrogen peroxide, NADH, and hydrogen radicals [~~singlet oxygen or hydrogen peroxide~~].

36. The set of reagent pairs of claim 35 wherein said sensitizer is capable of generating singlet oxygen when photoactivated.